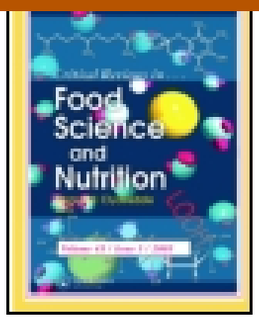

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Innovative ingredients and emerging technologies for controlling ice recrystallisation, texture and structure stability in frozen dairy desserts: A review

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ABSTRACT

Over the past decade, ice cream manufacturers have developed a strong understanding of the functionality of key ingredients and processing, developing effective explanations for the link between structure forming agents, stability mechanisms and perceived quality. Increasing demand for products perceived as healthier / more natural with minimal processing has identified a number of new tools to improve quality and storage stability of frozen dairy desserts. Ingredients such as dietary fibre, polysaccharides, prebiotics, alternate sweeteners, fat sources rich in unsaturated fatty acids and ice structuring proteins have been successfully applied as cryoprotective, texturizing and structuring agents. Emerging minimal processing technologies including hydrostatic pressure processing, ultrasonic or high pressure assisted freezing, low temperature extrusion and enzymatically induced biopolymers crosslinking have been evaluated for their ability to improve colloidal stability, texture and sensory quality. It is therefore timely for a comprehensive review.

Keywords: ice cream; cryoprotection; structuring; texture; stabilisers; texture; shelf-life; minimal processing

1. INTRODUCTION

Ice cream is a complex colloidal system that in its frozen state is comprised of ice crystals, air cells and partially coalesced fat droplets dispersed in a continuous freeze-concentrated aqueous phase containing solutes such as sugars, proteins, polysaccharides, and salts (Marshall et al., 2003). The formation of the structural elements and their thermodynamic stability during ageing are principally controlled by composition, freezing and post-hardening conditions. Ice cream manufacture involves several processing and handling steps a) formulation (ingredient blending, pasteurization, homogenization, cooling and ageing), b) freezing (scraped surface continuous or batch incorporation of air, flavourings and other inclusions), and c) post-freezing handling such as packaging, hardening and distribution (Marshall et al., 2003). Appropriate selection of ingredients together with customised processing can allow controlled colloidal quality and enhanced storage stability of ice cream (Goff, 1997). Over the last two decades, extensive research has been conducted to understand the

functionality of stabilisers and emulsifiers in model and real ice cream systems and to evaluate the potential of novel processing technologies to control of ice crystallisation and recrystallisation. The main effects of bulk ice cream components such as sugars, stabilisers and emulsifiers on ice formation and recrystallisation have been comprehensively reviewed (Bahramparvar and Mazaheri Tehrani, 2011; Cook and Hartel, 2010; Goff, 2002, 2008). In the present paper, recent advances in novel ingredient (polysaccharides, prebiotics and dietary fibre, bulk sweeteners and ice structuring proteins) and processing control (hydrostatic pressure, sonocrystallisation, transglutaminase cross-linking, low temperature extrusion) of ice cream structure and recrystallisation are reviewed.

2. CONTROL OF RECRYSTALLISATION DURING FROZEN DAIRY DESSERTS STORAGE: FROM EMPIRICAL TO MECHANISTIC APPROACHES

2.1 Ice formation and recrystallisation in frozen dairy desserts

Whilst ice crystallisation and recrystallisation both involve liquid to solid phase transitions driven by both heat and mass transfer, ice crystallisation is initiated by the formation of ice nuclei by undercooling, and ice recrystallisation is the growth of crystals via water diffusion at the liquid-solid interface (Hartel 2001). Ice crystallisation and recrystallisation in frozen dairy systems have been recently reviewed (Cook and Hartel, 2010; Hassas-Roudsari and Goff, 2012) and can be referenced for additional information. High nucleation rates (high $T-T_{fr}$ driving force and freezing rates) is very important for the formation of small crystals with narrow size distributions. Moreover, ice crystallisation impacts ice crystal growth during hardening and storage, and ultimately sensory quality drivers e.g. creaminess, iciness, coarseness, wateriness and hardness (Cook and Hartel, 2010; Muse and Hartel, 2004; Soukoulis et al., 2008; Soukoulis et al., 2010).

Notwithstanding the prominent role of ice crystallisation on texture development, ice recrystallisation governs texture and structure stability throughout post freezing operations. Ice recrystallisation associated with both dimensional and

morphological changes of ice crystals, is controlled by mass transfer (water diffusion), heat transfer (absorption or release of ice fusion enthalpy) and thermodynamic parameters (minimum free energy equilibrium state) (Hartel 2001). Extensive research conducted by the groups of Goff and Hartel elucidated the mechanisms of ice recrystallisation occurrence in ice cream as well as the cryoprotective role of polysaccharides and sweeteners. There are three main mechanisms that can be used to describe ice recrystallisation in ice cream: surface isomass, migratory and accretive ice crystal growth (Hartel 2001; Cook and Hartel 2010). Surface isomass recrystallisation is related with the achievement of a thermodynamically stable state by minimisation of the curvature of the ice crystals through surface diffusion. Migratory ice recrystallisation or Ostwald ripening is based on the growth of larger ice crystals in a polycrystalline system at the expense of smaller ice crystals (Hartel 2001; Zaritzky 2005). The cryoprotective action of several hydrocolloids has been related with the control of migratory ice recrystallisation (Regand and Goff, 2002b, 2003). Accretive ice recrystallisation occurs when two adjacent ice crystals join to form a single larger crystal. Contrary to migratory ice recrystallisation, accretion is not initiated by water diffusion from the bulk to the water-ice interfaces but

rather by surface diffusion across the crystal structure (Goff 2005). Accretive recrystallisation is reported to occur upon sugars and starch hydrolysates aqueous systems (Hagiwara and Hartel, 1996; Miller-Livney and Hartel, 1997).

2.2 Polysaccharides as cryoprotective agents: a mechanistic overview

The cryoprotective action of polysaccharides was previously reviewed by Bahramparvar and Mazaheri Tehrani (2011); therefore in the present work only a consise overview will be provided. Early explanations of the cryoprotective action of polysaccharides were based the reduction of water mobility due to macro-viscosity increase and the modification of colligative properties of ice cream mixes (Blond, 1986, 1988; Buyong and Fennema, 1988; Cottrell et al., 1979; Harper and Shoemaker, 1983; Muhr and Blanshard, 1984, 1986a, b; Shirai et al., 1985).

a) Micro-viscosity of the serum phase

In later studies, Hagiwara and Hartel (1996) and Miller-Livney and Hartel (1997) investigating the possible interrelationships between macro- and micro-viscosity of ice cream mix and unfrozen serum phase respectively, deduced that mix macro-viscosity is not associated with ice crystal growth under quiescent frozen

storage conditions. On the contrary, it was show that some colligative properties e.g. glass transition temperature and the amount of unfrozen water were related to the micro-viscosity of the freeze-concentrated micro-domain surrounding the ice crystals appeared to influenced the polysaccharides' cryoprotective activity. Regand and Goff (Regand and Goff, 2002a, 2003) showed that micro-viscosity could explain the cryoprotective action of some hydrocolloids such as xanthan and locust bean gum in model 40% w/w sucrose solutions which was attributed to their ability to retain water and form complex networks leading to sterically hindered water diffusion from the bulk serum phase to the ice crystals interface.

b) Viscoelasticity and critical viscosity of the serum phase

Dilute biopolymers in water will occur as individual or isolated polymer coil conformations, further increases in concentration will result in a reduction in the free hydrodynamic volume of the individual polymers and therefore biopolymers overlap and interpenetration can occur. The transition results in a breakpoint in the linear dependence of the apparent viscosity on biopolymer concentration (Phillips and Williams 2000). Goff (1995) and Bolliger et al., (2000c) studying stabilised model ice cream mixes, reported the existence of a critical percentage

of freeze concentration (c*) where a change in the slope of the linear dependence of serum micro-viscosity on solutes concentration occurs. Polymer entanglement in the unfrozen serum phase sterically hinders molecular mobility resulting in cryoprotection. Bolliger et al. (2000c) ENREF 8 successfully correlated c* with recrystallization, demonstrating that ice recrystallisation is minimised at the viscosity breakpoint.

c) Cryogelation

Muhr and Blanshard (1986b) observed that polysaccharide addition to sucrose solutions leads to the formation of cryogels which can inhibit ice recrystallization during temperature cycles. Moreover, gel forming stabilisers can impact ice crystal morphology and growth rate due to the ability of gel fibres to interact with ice crystals. Blond (1988) deduced that cryogels elasticity and ageing can reduce the recrystallisation rates though gel firmness does not necessarily imply cryoprotective ability, as the formed gels can be easily ruptured during ice front propagation. In a later study, Goff et al., (1999) showed that the cryoprotective ability of LBG in heat shocked sucrose solutions is associated with the formation of cryogels that entrap the ice crystals. This porous gel inhibits the collision

of ice crystals due to steric restrictions. Contrarily, the presence of stabilisers that do not form cryogels (e.g. guar) provide poor control of water diffusion from the unfrozen bulk phase to the ice crystals interface. Regand & Goff (2002; 2003) investigating the cryoprotective ability of cryogel forming polysaccharides (sodium alginate, xanthan, CMC, gelatin kappa-carrageenan and LBG), observed that in the absence of protein, only LBG was able to control ice recrystallisation rates compared to other gel-forming stabilisers e.g. gelatin and kappa-carrageenan. In addition, the development of firm and cohesive gels is not a prerequisite for cryo-stabilisation. The later was attributed to the ability of ice crystals to penetrate the firmest gels inducing syneresis and consequently increasing the molecular mobility of water in the micro-domains surrounding the ice crystals. Furthermore, Patmore et al., (2003) reported that viscoelasticity enhancement in protein-polysaccharides systems can hinder significantly the molecular mobility of water due to the resultant intermolecular interactions.

d) Phase separation

Biopolymers incompatibility known also as phase separation (segregative) refers to a thermodynamic - physical state where the solvent-biopolymer-biopolymer interaction is

favoured compared to biopolymer-biopolymer interactions (Corredig et al., 2011). As a result, a low phase separation threshold, which characterises proteins and polysaccharides, leads to the formation of liquid layers of low interfacial density (depletion) rich in each biopolymer. The phenomenon usually occurs under very specific biopolymer concentration and ionic strengths and can involve both charged and neutral macromolecules (Corredig et al., 2011). With respect to ice cream emulsions, phase separation is favoured in the presence of casein micelles and specific polysaccharides such as galactomannans or xanthan gum (Thaiudom and Goff, 2003). From a mechanistic point of view, phase separation in ice cream mixes is generally governed by depletion-flocculation of the casein micelles leading to the formation of a depleted layer around the micelles which due to osmotic pressure gradients forces them to attract with each other (Corredig et al., 2011). Phase separation is of particular importance for ice cream manufacturers as it can influence ice recrystallisation and on some occasions (e.g. soft serve ice cream mixes) is associated with undesirable defects. Secondary stabilising agents e.g. kappa-carrageenan, adjustment of the casein to whey protein

ratio, or modification of the primary stabiliser concentration (Spagnuolo et al., 2005; Vega et al., 2004; Vega and Goff, 2005) have been shown to control phase separation. Biopolymer concentration increases due to serum freeze-concentration diminishes the thermodynamic incompatibility of casein micelles and polysaccharides inducing depletion-flocculation. The formation of the protein and polysaccharides rich layers can contribute to the control of ice crystals growth through two mechanisms, the use of cryogel forming polysaccharides to control viscoelasticity and non-gelling biopolymers to reduce the molecular mobility of water in the bulk serum phase (Regand and Goff 2002,2003).

3 NEW CONCEPTS TO CONTROL RECRYSTALLISATION AND IMPROVING PERCEIVED TEXTURE QUALITY IN FROZEN DAIRY DESSERTS

3.1 Ice structuring proteins and ice nucleating proteins

3.1.1. Ice structuring proteins (ISP)

Antifreeze proteins (AFP) can be divided into two main categories: a) antifreeze glycoproteins (AFGP) and b) non-glycosylated antifreeze proteins. The latter are further

divided into ice structuring proteins (ISP) and thermal hysteresis proteins (THP). ISP are generally able to control the recrystallisation phenomena (size, shape and collision of ice crystals) whilst THP depress the freezing point of the solution (Clarke et al., 2002). The ability of ISP to control ice recrystallisation is explained by reference to the Gibbs-Thomson effect (the ice interfacial curvature induces a localised depression of the freezing point without affecting the bulk freezing point leading to thermal hysteresis). In aqueous systems, the AFP presence leads to the formation and growth of ice crystals following a needle-like or polygonal structural configuration. Raymond and DeVries (1977) suggested that below a specific temperature, AFP are adsorbed on the surface of ice crystals and inhibit molecular diffusion of water from the bulk aqueous phase to the ice crystal surface. The increase in the curvature of the ice crystals will therefore result in a concomitant reduction in recrystallisation rate according to the Gibbs-Thomson effect. Generally, the presence of low amounts of AFP promotes ice crystal growth along the a-axis (Fig.1), creating polygonal stereochemical configurations. Further increase of AFP concentration favours ice crystals growth along the c-axis

direction resulting needle-like conformations (Griffith and Ewart, 1995; Stressmann et al., 2004).

3.1.2 The use of ISP in ice cream

Fundamental research into ISP recrystallisation control in ice cream began almost simultaneously by Unilever (Byass et al., 1998; Lillford et al., 1998) and Pillsbury (Clemmings et al., 1997). Clemmings et al., (1997) demonstrated their potential in ice cream production without the need of hardening (1-10 ppm type I ISP). It was also shown that ISP containing ice creams had remarkably small ice crystals and low instrumental hardness, despite the hardening step absence. Similarly, Lillford et al., (1998) and Darling et al., (2001) added ISP type III (W097/02343) derived from Arctic fish or polar wheat grass (PCT/EP97/03634) before the freezing process, either directly or in solution in proportions ranging from 0.0005 % to 0.1 % (w/w). The researchers observed that presence of ISP at certain amount (0.01 % to 0.05 %, w/w) induces a reduction of ice crystals mean size providing satisfactory instrumental hardness. Jarman et al., (2005) isolated an AFP type III from cold acclimated grass using a high temperature (80°C – 105°C)

extraction process to produce an ISP product comprised mainly of Ser-Thr-Asp units that is characterized by resistance against heat induced protein denaturation and high cryoprotective action. The addition of 0.01 % (w/w) in ice cream improved stability under quiescent storage conditions, whereas the use of 0.001 to 0.05 % ISP was sufficient to inhibit ice crystals growth in sucrose solutions (30 % w/w) heat shocked at –6 °C for 1 h. Fenn et al., (2006) reported that an ice crystal's length to width ratio of 1.9 to 3 is associated with pronouncedly improved perceived texture. The addition of an APF type III HPLC-12 isolated from fish in conventional ice cream mix at the level of 0.01 % (w/w) induced the formation of ice crystals with a 2.24 length to width ratio compared to 1.45 of the control sample. The use of 0.005 - 0.05% (w/w) cold acclimated carrot juice isolated ISP in ice cream resulted in lower recrystallisation rates and narrower initial ice crystals mean size distributions without modifying the ice crystal morphological. The presence of carrot root isolated ISP led to the development of a harder and more brittle texture than conventional ice cream, although less hard than the ice creams stabilized with polar fish isolated ISP (Byass et al., 1998).

Regand and Goff demonstrated that the addition of winter grass ISP in ice cream significantly reduced the initial size of ice crystals (>44%) and inhibited recrystallisation after 16 consecutive cycles of melting - freezing. In addition, although the activity of ISP decreased during heat treatment of ice cream mixes, it remained sufficiently high to control ice recrystallisation (Regand and Goff (2006). From a mechanistic point of view, the cryoprotective action of ISP in both sucrose solutions and ice cream systems was attributed: a) diffusive migration of ISP molecules from serum phase to ice crystals interface, b) orientation into its low energy conformation on the ice crystal surface and c) diffusion around the ice crystal surface to find a specific location for incorporation into the ice lattice (Regand and Goff, 2005). However, the ISP ice recrystallisation inhibitory action can be more suppressed in ice cream mixes compared to sucrose solutions (Regand and Goff 2006). This was because of the antagonistic action between the ISP and other solutes (sugars, polysaccharides, proteins and salts) present in serum phase that can also diffuse from the liquid to the liquid-solid interface (Regand and Goff, 2006). The absence of any hydrocolloids was found to negatively

impact the ability of ISP to control ice crystal growth in heat shocked ice creams, this is possibly due to the hydrocolloid induced reduction in water molecules mobility (Regand and Goff, 2006).

Cationic polypeptides based on the primary sequence of collagen or gelatin with a molecular weight of 500-7000 Da have also been reported to exhibit remarkable antifreeze properties including ice crystal growth hindrance (Damodaran 2010). The enzymatic modification of gelatin with L-leucine n-dedecyl ester through the plastein reaction using papain has resulted in a hydrolysed derivative able to decrease the freezing point of water to about -7 °C in the presence of a heterogeneous nucleator inducer (Arai and Watanabe, 1986). Furthermore, in a series of studies Damodaran investigated the potential of using gelatin hydrolysed derivatives as ice crystal growth controllers in ice cream systems. Gelatin hydrolysed derivatives with molecular weights of 2000 – 5000 Da have shown outstanding ability to hinder ice recrystallisation in ice cream (Damodaran, 2007). The recrystallisation inhibitory activity of gelatin hydrolysates is not observed in larger fractions (> 7000 Da). Damodaran attributed the

antifreeze activity of gelatin hydrolysates to the formation of an oxygen triad plane at the C-terminal with oxygen-oxygen distances similar to those found in ice nuclei. The presence of this oxygen triad plane facilitates the binding via hydrogen bonds with the prism face of ice nuclei hindering the ice crystal growth in the ice cream mix (Damodaran, 2007; Wang and Damodaran, 2009) reported that the use of cationic polypeptide fractions (700 - 1400 Da) derived by papain gelatin hydrolysis at 30 – 37 °C , pH = 7 in ice cream mixes subjected to a freeze-thaw protocol (between –10 and –12 °C) reduced the ice recrystallisation rate by a factor of ten.

3.1.3 Ice nucleating proteins (INPs)

Gram negative bacteria such as *Pseudomonas syringae, Erwinia* and *Xanthomonas* are recognized as ice nucleating active (INA+) due to their ability to induce ice crystallization at low levels of supercooling e.g. at higher temperatures than the initial freezing point. These bacterial cells as well as their proteins (INP) can act as templates for the formation of ice nuclei. It has been reported that *Pseudomonas syringae, Erwinia ananas, Erwinia herbicola* and *Saccharomyches cerevisiae* release extracellular

material on the outer membrane that catalyses ice crystallization (Arai and Watanabe, 1986; Hwang et al., 2001; Zhang et al., 2009). INP are generally comprised of three distinguishable domain structures: a) a central repeating structural octapeptide unit (Ala-Gly-Tyr-Gly-Ser-Thr-Leu-Thr), b) a unique N-terminal hydrophobic domain and c) a unique C-terminal very hydrophilic domain rich in basic residues (Kawahara 2002). The INP nucleation activity is affected significantly by their structural conformation and size more than concentration (Zachariassen and Kristiansen, 2000). Moreover, parameters such as the INP size, quality and amount and the solutes concentration influence significantly the nucleation temperature (Zachariassen and Kristiansen, 2000).

Whilst many attempts have been made to the exploit the benefits of bacterial assisted ice crystallization (shorter freezing time, increased freezing rates, less texture damage of food tissues or cells, improvement of textural and thawing quality, reduction of refrigeration cost) in a wide range of food manufacturing applications (Li and Lee, 1998; Watanabe and Arai, 1994; Zhang et al., 2009, 2010), the actual applications of INP in real or model ice cream systems as nucleation promoting and ice crystallization

controlling agents is rather limited. Li and Lee (1998) reported that the addition of extracellular INPs from *Erwinia herbicola* in sucrose, protein and oil/water emulsions elevates the nucleation temperature and reduces the freezing point temperature without texture deterioration. Zhang et al., (2009) using a 10 % w/w sucrose aqueous system inoculated with ice nucleating *Pseudomonas syringae* (up to 1.38×10^5 cfu/g) , reported a remarkable reduction in the freezing time and elevation of the nucleation temperature, although the effects of INA+ bacteria on stable freezing point were rather negligible. In subsequent work, it was demonstrated that although the addition of INA+ bacteria in a highly concentrated (70% w/w) tylose solution elevated the nucleation temperature promoting the formation of uniformly distributed ice crystals, their presence did not influence the ice amount and glass transition temperature (Fig. 2). Contrarily, Hartel (1996) reported that ice cream mix inoculation with *Pseudomonas syringae* did not exert any significant changes in the ice crystals mean size distributions compared to the unseeded mixes.

3.2 Dietary fibre and prebiotics

The term dietary fibre refers to a heterogeneous mixture comprised of edible parts of plant cells, polysaccharides, lignin and associated compounds that are resistant to digestion and absorption in the human small intestine (AACC 2001). Dietary fibre has been associated with a wide range of physiological benefits and functional properties; these include a reduced risk of coronary heart disease, diabetes, obesity, and some forms of cancer, lowering of blood lipids level and prevention of bowel disorders (Westenbrink et al., 2013). Moreover, dietary fibre can exhibit interesting techno-functional characteristics in products e.g. water and oil binding capacity, good emulsifying activity, gel forming ability, thickening and texturisation rendering them valuable components in ice cream for their ability to form texture and stabalise the matrix (Elleuch et al., 2011). Polysaccharides such as cellulose, pectin, algae and plant extracts are the predominant sources of dietary fibre used in ice cream. However, their relatively low amount in ice creams (< 0.5 % w/w) is not adequate to meet the recommended FAO/WHO dietary fibre daily intake levels (~25 g / day). The use of prebiotics such as inulin, fructo-oligosaccharides (FOS), or polydextrose at substantially

higher amounts than conventional hydrocolloids permits the fortification of ice cream with soluble and insoluble fibre, increasing its nutritional value and in many cases improving its textural characteristics and storage stability (Akalin and Erisir, 2008; Karaca et al., 2009; Schaller-Povolny and Smith, 1999; Soukoulis et al., 2009; Soukoulis et al., 2010).

FOS have been extensively tested both as ice recrystallisation controllers and texture enhancers. Schaller-Povolny & Smith (2001) studying the potential of inulin as a corn syrup solids (42DE) replacer and reported a significant elevation of the freezing point and enhancement of the viscosity of ice cream mixes. Similarly, Soukoulis et al. (2009; 2010) scrutinising the impact of inulin and oligofructose supplementation (2 - 4 % w/w) on ice crystallisation and recrystallisation in model ice cream systems reported a significant elevation of the glass transition, ice fusion and freezing point temperatures due to steric hindrance of the water molecules in the serum phase. Moreover, the FOS containing ice cream systems were characterised by a narrower ice crystal size distribution imparting a smoother, creamier and less watery perception.

The addition of FOS in ice cream mixes has been reported to also modify their rheological properties due to their ability to bind water, although this is dependent on their degree of polymerisation and branching (Soukoulis et al., 2009; Soukoulis et al., 2010). In most cases, the addition of inulin increased the shear thinning behaviour of ice cream mixes as well as the viscosity at a shear rate corresponding to oral processing e.g. 50 s^{-1} (Soukoulis et al., 2010). Similar results were also observed in fermented ice creams as reported by El-Nagar et al. (2002), Akalin and Erisir (2008) and Akin et al. (2007). In addition, the use of inulin at high amounts (> 5 % w/w) was reported to increase the instrumental and organoleptically perceived hardness and to reduce the melting rate. This was attributed to its impact on the colligative properties of the systems, water retention and gel forming ability (Akalln et al., 2008; Aykan et al., 2008; El-Nagar et al., 2002; Karaca et al., 2009; Soukoulis et al., 2010).

Soluble and insoluble dietary fibres isolated from plants (i.e. vegetable, fruits, cereals and pulses) have been also evaluated in ice cream due to their peculiar physiological, nutritional

aspects technological characteristics (Bahramparvar et al., 2010; Chen et al., 2010; Cody et al., 2007; de Sales et al., 2008; Dervisoglu, 2006; Dervisoglu and Yazici, 2006; Soukoulis et al., 2009; Sun-Waterhouse et al., 2013). Soukoulis et al., (2009) demonstrated that oat, wheat or apple fibre fortification of both sucrose solutions and ice cream mixes impart a remarkable cryoprotective action by elevating the glass transition and freezing point temperatures. The changes of the thermophysical properties were mainly associated with restricted molecular mobility of water molecules induced by the water binding of the insoluble matter that leds to enhanced freeze concentration of the serum phase. Moreover, in the case of apple fibre, pectin induces phase separation promoting cryoprotection, an action that was not evident in the absence of milk proteins. Chen et al., (2010) used soluble soybean polysaccharides (SSPS) a soy manufacturing by-product isolated from okra, as a fibre fortifying (added at the level of 1-3% w/w) and structuring agent in fat-free ice cream formulations. SSPS presence was accompanied by a significant increase of the mixes viscosity and a remarkable synergistic action with guar gum and kappa-carrageenan, though no significant differences in the

firmness and meltdown were observed. Dietary fibre derived from fruit pulp or flesh has been also tested for its impact on ice cream texture and structure stability. Dervisoglu and Yazici (2006) demonstrated that citrus fibre (ranging from 0.4 to 1.2% w/w) cannot fully substitute stabilisers due to their low impact on rheological and textural properties and overrun. However, in the presence of stabilisers the fibre exhibited a notable synergistic action leading to viscosity, overrun, texture and melting resistance enhancement.

The use of cereal flours has been also proposed as a strategy to improve textural and melting quality of ice creams. Cody et al., (2007) showed that the addition of rice flour in regular (10% milkfat w/w) and non-fat ice creams constrained recrystallisation throughout frozen storage based on a descriptive analysis basis. Increasing the rice flour solids from 2 to 6 % w/w resulted in a significant decrease of the perceived iciness and coarseness, whilst it improved creaminess, gumminess and chewiness. Apart from flour off-flavour none of the taste-flavour descriptors including vanilla flavour intensity were influenced in rice flour presence. In addition, its functionality was not affected by

the thermal history of the ice creams e.g. thermal abuse (Cody et al., 2007). Hazelnut flour (1 to 4.5 % w/w) and skins (1 to 3 % w/w) have been also studied for their impact on the textural quality and meltdown of fat-free vanilla ice cream (Dervisoglu, 2006). Although both ingredients improved the viscosity, overrun and melting resistance of the ice cream systems, they deteriorated colour and texture characteristics.

The desirable flavour characteristics of fruit together with their nutritional characteristics e.g. natural sources of dietary fibre and phytochemicals have drawn attention to their incorporation in ice cream. Recently, Sun-Waterhouse et al. (2013) studied the effects of kiwi pulp from three different cultivars (red, green and gold) in ice cream, as a vehicle for delivering targeted functional aspects (e.g. increased antioxidants) and technological properties. All kiwi pulp containing ice creams exhibited a viscoelastic behaviour with storage modulus G' being cultivar dependent e.g. gold flesh pulp addition led to the stiffest structure. Similarly, melting resistance and overrun of ice creams increased according to the order of red>gold>green indicating a higher percentage of fat destabilisation and structure reinforcement in the case of red flesh kiwi fortified ice cream. The addition of gooseberry pulp

in ice cream as a strategy for natural fortification of the antioxidants present in ice cream was investigated by Erkaya et al., (2012). Increasing gooseberry total solids in ice cream enhanced mixes viscosity and ice cream melting resistance due to the high water holding capacity of the present dietary fibre. No adverse effects on organoleptic quality and overrun were detected. Grape wine lees (GWL) were also successfully used as natural antioxidant sources in ice cream (Hwang et al., 2009). GWL addition in ice cream improved viscosity, melting resistance and fat destabilisation rates, and reduced the percentage of unfrozen water. The ability of the soluble and insoluble fibre to uptake and bind water was associated with the observed effects on rheological and thermal properties. Notwithstanding the improved free radicals scavenging action, the concentration of GWL in ice cream determines the detrimental impact on overrun, flavour and colour, with 5% w/w as being the threshold for ensuring acceptable quality traits.

Over the last years, the use of exudates from endemic plants as novel stabilising systems has also gained a lot of attention. Bahramparvar and others have investigated

the addition of a soluble fibre isolated by basil seeds as an alternative ice cream stabiliser (BahramParvar and Goff, 2013; BahramParvar et al., 2012). The presence of basil seed (*Ocimum Basilicum L.)* gum in ice cream systems, induced a significant increase of the mixes viscosity, improved the melting resistance of the finished product and also facilitated air incorporation during the whipping-freezing process (BahramParvar et al., 2012)). Recently, BahramParvar and Goff (2013), demonstrated that the presence of basil seed gum in thermally abused ice creams was able to reduce the rate of ice crystals growth by 30-40% compared to other commercial stabilisers e.g. CMC or guar gum. Moreover, different ice cream structures were identified in the case of the basil seed gum stabilised systems, due to a higher extent of fat droplet coalescence, air cells development and stabilisation, attributed mainly to the ability of basil seed gum to significantly lower the air and fat interfacial tension. The latter also explained the improved melting resistance and shape retention of the systems containing basil seed gum. In another study, Bahramparar (2010) investigating the impact of Lallemantia royleana (Balangu) seed gum and palmate-tuber salep gum

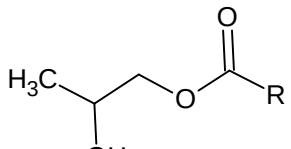
reported that Balangu seed gum exhibited a similar ability to enhance viscosity, favour air incorporation and controlled meltdown and retain texture stability upon storage compared to that of CMC. Kaya and Tekin (2001) observed a shear thinning behaviour of ice cream mixes containing salep (*Orchis orchida*), with viscosity being significantly dependent on salep content. Although in a later study, Guven et al. (2003) found that the use of salep as individual stabiliser in ice cream does not provide sufficient cryoprotection to the ice cream mix, leading to harder, coarser and more brittle structures compared to those stabilised using CMC, sodium alginate, locust bean or guar gum.

3.3 Propyleneglycol monostearate

Propyleneglycol monostearate (PGMS) was introduced as an emulsifying agent in several aerated bakery products and powdered toppings. Its functionality is generally associated with its peculiar crystalline properties and its ability to stabilise the meta-stable α-crystal form of monoglycerides thereby enhancing the synergistic interactions

with other emulsifying agents and thus, inducing the interaction with water molecules and gel formation at low temperatures (Sparsø & Krog 2004).

The use of emulsifiers that facilitate the formation and stabilisation of fat α-crystals as alternative cryoprotective agents in ice cream has been established (Aleong et al., 2008). Vaghela et al., (2013) patented a process for ice cream production with high resistance against heat shock comprised of at least one emulsifier of the α-crystalline form such as PGMS, sorbitan tristearate (STS), lactylated (LACTEM) or acetylated monoglycerides (ACETEM). The product is characterized as having reduced air cell and ice crystal mean sizes (less than 50 and 25µm respectively), improved creaminess, texture uniformity and mouth coating. In 2004, Barfod and others have used PGMS as an ice crystal growth inhibitor both during the stages of freezing and hardening-storage. In their studies, Barford et al., (2009) have demonstrated that the use of compounds with the chemical formula (I) provide a stronger thermodynamic barrier against ice crystal growth.



(I)

PGMS interacts with the partially coalesced fat droplets via the β-structure fat phase stabilisation mechanism, leading to the formation of a lamellar phase on the fat droplets surface. This thin layer covering the ice crystals interface is able to interact with water molecules inhibiting their diffusion to the ice crystal surface. Although, STS, LACTEM or ACETEM do not hinder the ice crystals growth during heat shock, their presence in small amounts might contribute to the enhancement of air incorporation and improve the air cells stability throughout storage. Indeed, the presence of PGMS alone had a detrimental effect on air incorporation upon whipping and shape retention and shrinkage control in thermally abused samples. Aleong et al., (2008) elucidated the mechanisms underlying the cryoprotective action of PGMS in model sucrose and ice cream systems. PGMS caused a significant reduction in the average size of both the initial ice crystals and ice crystals formed after successive freeze-thaw cycles. It is notable that the addition of PGMS reduced the average size of

ice crystals by 50% compared to samples containing conventional stabilisers. Moreover, PGMS appeared to control the growth of ice crystals and size during freezing, when the impact of conventional stabilisers is very limited (Caldwell et al., 1992) or negligible (Sutton and Wilcox, 1998a, b). PGMS can also contribute to texture improvement as ice crystals mean size distributions similar to those of low temperature extruded ice cream have been identified (Barford et al., 2005). Aleong et al., (2008) demonstrated that the presence of PGMS alters remarkably the morphology of ice crystals (Fig. 3). The ice crystals in PGMS containing systems exerted a heavily sculpted and irregular shape, unlike the uniform and smooth ice crystals in the case of samples without PGMS. Moreover, the lipophilic nature of PGMS promotes ice crystals interaction with the destabilized fat globules as indicated by the formation of PGMS α-crystals on the surface of ice crystals increasing the radius of curvature and surface free energy of ice crystals. The Gibbs-Thomson theory that describes the action of antifreeze proteins has been proposed to explain the action of PGMS. Doxey et al. (2006) reported that the cryoprotective action of antifreeze proteins is potentially associated with their connection with the ice crystal in positions where

apolar functional groups compatible with the surrounding solvent interface can be found (e.g. methyl groups). Indeed, PGMS carries methyl groups which act as binding centres for the water molecules leading to the formation of crystalline structure on the surface of the ice crystals (Wierzbicki et al., 2007). Moreover, considering that PGMS functionality is associated with its distribution on ice crystals surface, high shearing freezing processes (e.g. low temperature extrusion) generally enhance its cryoprotective role (Aleong et al., 2008).

3.4 Trehalose as sucrose substitute

α-α-Trehalose is a naturally occurring disaccharide consisting of two glucose moieties linked through their respective anomeric carbon atoms by an alpha-glycosidic bond (O'Brien-Nabors, 2011). Major sources of trehalose are bacteria, fungi, yeast and algae though it is also present in foods such as mushroom, honey, bread and fermented beverages. Trehalose has a clean sweet aftertaste, with almost half the sweet intensity of sucrose, it does not undergo thermal or hydrolytic breakdown over a broad pH range and is not subject to Maillard reactions. The glass transition temperature of trehalose

(Tg ~ 120°C) is significantly higher compared to sucrose or maltose leading to a remarkable thermo- and cryo-protective action (Cesàro et al., 2008).

Whelan et al. (2008) investigated the effects of trehalose on the glass transition phenomena and ice growth during accelerated shelf-life in model solutions and ice creams containing sucrose. Trehalose induced a 6.5 and 4.4°C elevation of glass transition temperature in the aqueous and ice cream systems compared to those containing sucrose. However, the partial substitution of sucrose by trehalose did not modify the ice crystal mean size distributions of thermally abused systems. Soukoulis et al. (2010) have investigated the effect of several sweeteners including trehalose on the thermal, rheological and sensory characteristics of ice creams. The partial substitution (30%) of sucrose did not modify the rheological parameters (consistency coefficient and rheological behaviour index) whilst it led to a significant elevation (~2°C) of the glass transition temperature, overrun and melting rate values. Trehalose improved perceived texture (icy, coarse, and creamy) and sweetness of ice creams.

3.4 Fat sources rich in unsaturated long chain fatty acids

It is well established that physicochemical and thermal properties of fat are key factors for the development of structural and textural elements of ice cream. During the freezing-whipping process the fat crystals found within the oil droplets move and reside at the oil-water interface leading to the penetration and rupture of the oil droplets surface, and the formation of fat clumps that retain partially their initial structural identity but are intricately linked (Goff, 1997). The clustering of fat droplets permits the formation of a three-dimensional network that immobilises and stabilises colloidal structures (air cells or ice crystals), improves melting resistance and shape retention, and imparts important organoleptic features such as creaminess, mouth coating and tongue lubrication (Goff, 1997; Sofjan and Hartel, 2004). The extent of partial coalescence during ice cream manufacture is influenced by many factors including milk fat droplets membrane composition, presence of amphiphilic surfactants, amount of crystallised fat and the mechanism (orthokinetic or perikinetic) of fat aggregation (Goff, 1997; Marshall et al., 2003).

In conventional ice cream the use of milk fat provides adequate crystallised fat to promote partial coalescence and impart good organoleptic characteristics (Marshall et al., 2003). The desire by consumers for healthier ice cream has encouraged the replacement of milk fat with vegetable fats and oils. However, in many cases milk fat substitution is accompanied by alterations to fat destabilisation, colloidal stability, melting resistance and sensory traits. Control of the amount of crystallised fat is crucial for structure and texture development of ice cream (Goh et al., 2006; Sung and Goff, 2010). More specifically, the use of a vegetable fat blends comprising palm kernel and sunflower oil with a solid to liquid fat ratio ranging from 60 to 80% has been noted for its efficacy to facilitate fat destabilization, enhance partial coalescence, and strengthen melting resistance and structural integrity. Intermediate solid fat index (SFI) values can permit protein displacement from the fat droplets surface thereby promoting partial coalescence, whilst a decrease of the SFI seems to favour the dominance of coalescence against partial coalescence due to the presence of oil-rich droplets. Although the saturation level of the fat source might lead to

significant differences in the SFI it is not always the driving factor of partial coalescence as changes to the saturation level of emulsifiers also play a significant role. Granger showed that the degree of saturation of the surfactants can dominate the formation of fat aggregates with unsaturated oil soluble emulsifiers being remarkably reactive with solid fat (Granger et al., 2005a, b). As a result the fat crystals that are gradually growing distort from the spherical to platelet-like shape conformations (Barfod et al., 2000). Moreover, the mean size of fat aggregates in whipped ice cream exhibited a significant dependence when unsaturated emulsifiers were used, palm kernel and coconut oil were more susceptible to partial coalescence than anhydrous milk fat despite the complete fat crystallization of the latter (Granger et al., 2005). Recently, Méndez-Velasco and Goff (2012a, b) elucidated the synergistic interaction between emulsifier saturation level and solid fat amount in ice cream systems containing binary blends of palm kernel oil with high oleic sunflower oil (HOSO). The authors observed that the presence of HOSO affects the structural conformation of the coalesced fat droplets favouring the formation of platelet-like shapes upon ice cream hardening (Fig. 4). In the case of the fresh frozen

unhardened ice creams the emulsified liquid fat is more prone to coalescence instead of partial coalescence which was confirmed by the development of platelets clusters. On the other hand, the use of unsaturated emulsifiers in ice cream mixes containing HOSO can induce an increase in the fat particles mean size and strengthening of the storage module (G') of the aerated structure (Méndez-Velasco and Goff, 2012a). Moreover, the solid fat content and the emulsifier type (saturated or unsaturated) driving the mechanisms of fat destabilisation e.g. partial coalescence, coalescence, protein induced flocculation or their combinations (Velasco and Goff 2012b).

Goh et al., (2006) incorporated flaxseed oil rich in alpha-linolenic fatty acids into ice cream and reported a decrease of instrumental hardness and melting resistance mainly attributed to poor partial fat coalescence. The authors investigating the colloidal properties of the melted ice creams in the presence or absence of SDS/EDTA observed only minor changes in the fat droplets mean size distributions between milk fat and flaxseed containing systems. Although, partial coalescence was the dominating structure stabilizing mechanism for ice cream containing up to 75% flaxseed oil, the

coalescence of liquid fat droplets was detected using CLSM in the ice creams containing exclusively flaxseed oil. Modification of the solid to liquid fat ratio also affects the rheological behaviour of the ice cream systems upon shearing and their structural recovery at the end of the mechanical treatment (Bazmi and Relkin, 2009). The elevation of olein to stearin ratio seems to favour the structural recovery of aged ice cream emulsions facilitating the interaction of fat droplets with other colloidal elements present e.g. proteins or polysaccharides and consequently to facilitate air incorporation and air cells stabilization. Modification of the fat crystal network of the bulk oil e.g. via inter or trans-esterification has been also reported as an important factor that may lead to changes of the rheological properties and emulsion and microstructural stability. In their study, Liew et al. (2001) reported that the trans-esterification of palm kernel oil increased the complex viscosity, storage modulus (G') and emulsion stability of 10% w/w fat containing ice cream mixes due to changes of the interfacial film surrounding fat globules and the interactions and crosslinking between proteins and fat droplets. These

authors concluded that the partial substitution (30% w/w) of anhydrous milk fat by trans-esterified palm kernel oil could be successfully applied in the ice cream manufacture.

Investigating the feasibility of palm oil fractions and rice bran oil in ice cream manufacture as a healthy analogue of milk fat, Nazaruddin et al. (2008) have concluded that the addition of 12% w/w of rice bran oil in ice cream can improve significantly its quality characteristics (smooth and soft texture) without acting against structural integrity and stability. Im and others showed that ice cream with elevated amount of mono and poly-unsaturated fatty acids (SFA:MUFA:PUFA ratio 3:2:1) supplemented by canola and soybean oil was equally scored equally to regular vanilla ice cream (SFA:MUFA:PUFA ratio 24:8.6:1) in terms of smoothness, mouth-coating, stickiness and melting quality without imparting any off-flavour, aftertaste or oxidation defects (Im et al., 1994). However, the proper design of the fat source formulation was found to be a critical factor for constraining any undesirable defects such oiliness, rancidity, coarseness, or poor melting resistance. Time-intensity (TI) flavour profile of ice creams with dairy or vegetable oil has revealed only a minor effect of the fat type on the flavour

release rate whilst maximum intensity and total aroma delivered did not differ among formulations (Hyvönen et al., 2003). The same authors reported that the complete substitution of dairy fat with vegetable did not modify the aroma and flavour intensity, sharpness, typicality, perceived sweetness, fattiness and creaminess.

3.5 Emerging processing practices for controlling ice nucleation

Although most of the cryoprotectants used in ice cream manufacture aim to the control of ice crystals growth during the frozen storage, novel processing practices such as high pressure and ultrasound assisted freezing can impact the initial mean size of ice crystals. This is attained by controlling ice nucleation during the initial phase of the freezing process (Chevalier et al., 2000; Kiani and Sun, 2011; Volkert et al., 2012).

3.5.1 High pressure low temperature treatment

Ice crystallisation is a two-stage process: nucleation and ice crystal growth. In ice cream production the mechanisms describing nucleation and ice crystal formation are still somewhat elusive; the high temperature gradients achieved in the scraped surface freezers promote the formation of dendritic or needle-like primary ice nuclei on the freezer's walls. As the

freezer blades are scraping the frozen material the dendritic ice crystals are broken down and transferred to the warmer bulk ice cream mix phase causing their ripening into a disc-like conformation (Cook and Hartel, 2011). The operational conditions of the freezer (dasher size and speed; drawing temperature), coolant and residence time are known to influence ice nucleation and growth (Drewett and Hartel, 2007; Russell et al., 1999).

High pressure shift freezing (HPSF) has been applied initially to cryo-immobilise samples for microscopic observation. For food applications, HPSF was introduced as an alternative process for the production of frozen tofu cheese and dairy gels (Fuchigami et al., 2002; Fuchigami and Teramoto, 2003). During HPSF an aqueous system is pressurised in the liquid state and then cooled down to temperature below its atmospheric pressure freezing point without any ice formation. When the desired subzero temperature is achieved, the pressure is released prompting a rapid supercooling that induces the formation of ice nuclei uniformly distributed throughout the product regardless its geometrical or morphological aspects. In a successive step, the freezing process is accomplished under atmospheric pressure conditions (Chevalier et

al., 2000; Fernández et al., 2006; Otero and Sanz, 2006). The formed ice crystals using HPSF are characterised by low mean diameter and granular shape thus improving texture and storage stability of final product whilst ensuring minimal disruption of cellular tissues and modification of the microstructural elements (Martino et al., 1998; Urrutia et al., 2007; Zhu et al., 2005). Fernández et al. (2007) investigated the synergistic effect of HPSF and cryogel forming hydrocolloids on ice crystallization in model sucrose solutions. The use of HPSF led generally to the formation of significantly smaller ice crystals compared to conventional freezing due to greater supercooling and shorter phase transition times. Moreover, it was deduced that HPSF does not show any modification of the cryogel forming hydrocolloids and therefore acts synergistically in terms of cryoprotection. In a recent study, Volkert et al., (2012) have investigated the effect of High Pressure Low Temperature (HPLT) on the freezing behavior and textural properties of model frozen foam and unfrozen emulsions in the presence or absence of air. HPLT freezing of the liquid emulsions is achieved through the ice liquid metastable to ice I nucleation (at the pressure range of

50 to 100MPa) – crystallisation, whereas HPLT treatment of frozen foam follows the ice I – ice III – ice I solid metastable phase pattern. The HPLT treatment of both liquid and solid aerated systems has a rather negative effect on their air volume fractions (shrinkage) as a consequence of the pressure release. Shrinkage was found to be dramatically higher in the case of frozen foam due to the transition of air from supercritical droplet (under high pressure conditions) to gaseous state upon pressure release. However, HPLT does reduce remarkably the ice crystal means size resulting in a thermodynamically stable product with improved organoleptic characteristics e.g. smoothness and mouth coating.

3.5.2 Ultrasound assisted ice crystallization

Power ultrasound is an emerging technology in the food processing industry with its main applications restricted to cleaning of processing equipment, inactivation of pathogenic microorganisms and enzymes in food products, emulsification and size reduction, extraction of bioactive compounds from natural sources, reduction of viscosity of food products, improvement of whey ultrafiltration yield, lactose recrystallisation and more recently inducement of ice crystallisation during conventional

freezing process of food (Ashokkumar et al., 2010). The feasibility of ultrasound assisted freezing (sonocrystallisation) is mainly associated with the formation of cavitation bubbles that act as ice nuclei, increase of heat and mass transfer and the breakdown of dendritic ice crystals leading to a more uniform ice crystals distribution (Kiani and Sun, 2011). Acton and Morris (1992) patented a technology for ice cream ultrasonic assisted freezing by installing an ultrasonic inducer at the external of a scraped surface ice cream freezer. Sonocrystallisation throughout the freezing-whipping process prevents the encrustation of ice crystals on the freezing barrel surface improving the performance of the dasher and facilitating the formation of uniform tiny ice crystals. The same authors highlighted that the reduction of the overrun occurred due to the degassing effect of ultrasound cavitation can be surpassed by either increasing the percentage of the incorporated air or the operating freezer pressure. Mortazavi and Tabatabaie (2008) reported that the exposure of ice cream mixes to acoustic treatment (up to 60 min at 20 kHz) prior to ageing was accompanied by a significant reduction of the ice cream freezing time from 20 to 12 min. A 20 min ultrasonication of the ice cream

mixes was found to minimise the formation of off-flavours, maximise consumer acceptability and overrun values of the finished frozen products. The enhanced air incorporation observed in the case of sonicated ice cream systems was attributed to occurrence of compression-rarefaction cycles leading to the formation of cavitation air cells (Mortazavi & Tabatabaie 2008). Similarly, Jambrak et al. (2012) while investigating the colligative properties and whipping ability of ultrasound treated ice cream mixes reported that the prolonged sonication of ice cream results in improved air incorporation and induces a significant depression of the freezing point temperature. Thus, a proper selection of the sonication conditions is critical to prevent the adverse impact of ultrasonication on ice crystallisation phenomena e.g. accumulated thermal energy due to prolonged acoustic exposure of the ice cream systems.

3.6 Low temperature extrusion (LTE)

Low temperature extrusion (LTE) is a relatively new technique in ice cream manufacture and was implemented as a development of the conventional freezing process i.e. the

ice cream enters the LT extruder at -5°C and leaves it approximately at -12°C. The LTE of freshly frozen ice cream induces a remarkable size reduction of ice crystals and air cells as a result of the high shear stresses applied (Wildmoser 2004). This can lead to a significant improvement of the perceived creaminess, increase the scoopability, cohesiveness and shape retention. Moreover, LTE allows the increase of the percentage of frozen water (ice) without inducing significant growth of the ice crystals and thus, it can eliminate the need of conventional tunnel hardening.

The impact of LTE on the structural elements of ice cream have been scrutinised in several studies (Bolliger et al., 2000b; Eisner et al., 2005; Wildmoser et al., 2004). The application of high shear forces on the viscoelastic matrix induces the partial coalescence of the primary fat globules due to the removal of the milk fat globules membrane. The percentage of the fat droplets destabilisation in the LTE treated systems can be twice that achieved during the continuous freezing-whipping process thereby reducing the amount of emulsifier required (Fig. 5). The LTE

treated ice creams had a greater melt resistant due to the higher extent of the fat destabilisation achieved (Eisner et al., 2005).

The ice crystals size reduction that takes place during LTE is associated with the control of ice crystal growth due to the applied high shear forces, the rapid freeze concentration of the serum phase, the reduction of the water diffusion rates and the hindrance of the collision of the adjacent ice crystals (Bolliger et al., 2000a; Bolliger et al., 2000b; Wildmoser et al., 2004). The increase of serum micro-viscosity as result of the freeze concentration combined with the high shear forces can also lead to a remarkable reduction of the air cells. The latter increases the thermodynamic instability of air cells due to high Laplace pressures via the Ostwald ripening pathway. However, it has been reported that even under these conditions shrinkage rate during frozen storage in LTE treated ice cream is rather low compared to conventional products (Eisner et al., 2005).

3.7 High hydrostatic pressure pre-treatment of ice cream mixes

High hydrostatic pressure (HHP) processing is a relatively new process that, depending on application, is proposed to reduce processing of food products, extend of the shelf-life, improve quality and nutritional value (Trujillo et al., 2002). Over the last decade, a large number of applications related with the implementation of HHP in the manufacture of several dairy products such as milk, cheese, yogurt, fermented milks, cream, butter and ice cream have been reported (Trujillo et al., 2002). In ice cream manufacture, the feasibility of HHP is mainly associated with its impact on the solid-liquid phase transitions of water and its ability to induce the partial denaturation of whey proteins providing an alternative strategy for structure and texture improvement (Huppertz et al., 2006; Trujillo et al., 2002). The use of HHP in the production of aerated dairy emulsions is related with the disruption of intermolecular hydrophobic and electrostatic interactions between whey proteins that increase their surface hydrophobicity and their ability to lower the interfacial tension and protein adsorption on the interface of fat droplets promoting their partial coalescence, enhancing foaming ability and physical stability (İbanoğlu and Karataş, 2001; Lim et al., 2008a). The latter may have a significant

impact on the colloidal aspects and the thermodynamic stability of the structural elements e.g. air cells, fat droplets and ice crystals. Lim et al. (2008b) observed that HHP treatment of whey protein concentrate stabilized ice cream mixes led to a significant increase of their foaming ability and overrun, and led to a stronger pseudoplastic behaviour as induced by the partial denaturation of the whey proteins. In addition, the partial exposure of the hydrophobic domains of the proteins facilitated the contact between the water-associated hydrophobic groups (namely hydrophobic hydration water) and the freeze-concentrated serum phase leading to harder products that the conventional ones (Lim et al., 2008b). TEM of ice cream mixes treated with HHP reveals the presence of casein micellar fragments densely packed within the serum phase (Fig. 6). The aggregation of the micellar casein fragments is hindered by the formation of HHP induced whey proteins denaturation whereas is promoted in the presence of fat and carbohydrates due to the increase of the casein to water ratio (Huppertz et al., 2011). These changes were determined to be the drivers of the enhancement of mix viscosity and resistance against meltdown (Huppertz et al., 2011).

Thus, the implementation of HHP in ice cream manufacture could be a promising strategy for the production of high quality low fat or stabilizer-free ice cream. The ability of HHP to induce β-lactoglobulin into the molten globule state can also potentially lead to modification of the binding affinity for several volatile flavour compounds. (Chauhan et al., 2010) demonstrated that diacetyl levels in the headspace above at HHP whey protein concentrate stabilized ice creams were 3-fold higher after 1 and 7 days of frozen storage. However, diacetyl intensity was perceived at the same pattern by the consumers for both the control and HHP treated samples implying that the effects of HHP on flavour quality of ice cream are only minor.

3.8 High pressure homogenisation and microfluidisation

Homogenisation is associated with fat droplet size reduction, modification and redistribution of the surfactant molecules in the MFGM interface. This leads to better foaming capacity and increased fat droplet stability throughout ageing (Goff 1997). High pressure (HPH) and ultra-high pressure (UHPH) homogenisers operating at pressures

up to 200 MPa have been successfully for the production of a broad range of dairy products such as milk, dairy emulsions, yogurt and cheese (Dumay et al., 2013). Size reduction, control of phase separation emulsification, partial protein denaturation and modification of biopolymer's structural conformation have been reported to occur by the implementation of HPH/UHPH (Dumay et al., 2013). Innocente et al. (2009) found that HPH of ice cream mixes acts mainly on the colloidal phase leading to a strong enhancement of the viscoelastic properties and apparent viscosity. Rheological characterisation of both HPH and conventionally homogenised mixes unveiled that HPH treated systems exhibited a strong gel-like behaviour which was mainly attributed to the formation of an interpenetrating protein network where fat globules reside internally acting thus, as active fillers. The impact of fat globule size reduction for conventionally homogenized mixes is generally more pronounced compared to HPH treated ones, due to the minor impact on the colloidal protein material. Hayes et al. (2003) demonstrated that HPH treatment of ice cream mixes can induce fat coalescence which is more prominent as the content of milk fat increases

leading to changes of the structural elements of the finished frozen product. More specifically, it was shown that HPH enhances the textural qualities of low-fat ice cream exhibiting similar gumminess and hardness scores to those of regular ice creams. A two-successive step homogenisation at conventional pressures e.g. 13.8 and 3.5 MPa has been reported as an alternative strategy for texture and melting quality improvement. Double homogenized ice creams, with lower amounts of stabiliser added, had smaller mean sizes of ice crystals and greater mouth coating and creaminess (Ranjan and Baer, 2005; Ruger et al., 2002).

Similarly to HPH, microfluidisation is based on forcing a fluid to pass under extreme pressure (150-250 MPa) through an interaction chamber with fixed geometry consisting of two parallel channels which make an abrupt 90^{o} turn and feed into a single channel, microfluidisation can also applied for tailoring the colloidal aspects of ice cream. The microfluidisation of ice cream mixes at pressures ranging from 50 to 200 MPa has been reported to improve the melting resistance of the finished product compared to the conventionally homogenised ones (13.8 and 3.4 MPa). High pressure homogenisation

favoured the coverage of fat globules by casein micelles as well as the aggregation of the proteinaceous material thereby improving melting quality without changing the flavour and textural properties of ice cream (Olson et al., 2003). Cavender and Kerr (2013) testing the impact of microfluidisation (at 220-250 MPa) of ice cream mixes contained xanthan or locust bean gum demonstrated that ice creams made of microfluidised emulsions were characterised by improved sensory (creaminess and firmness) and textural (adhesiveness and gumminess) properties depending also on the stabilising system. However, in the same study, the implementation of a microfluidisation step did not influence significantly their melting resistance.

3.9 Transglutaminase assisted protein cross-linking of the ice cream mixes

Transglutaminase (TGase, EC 2.3.2.13) is a transferase that catalyses the reaction between the γ-carboxyamide groups of peptide bound glutamyl residues (acyl donor) and several primary amines including ε-amino group of lysine leading to protein cross-linking through the formation of both inter- and intra-molecular isopeptide bonds (Motoki

and Seguro, 1998; ÖZrenk, 2006). The affinity of TG with different proteins relies on the distribution of the glutamine residues and their structural conformation as well. Non-globular milk proteins such as α-, β- and κ-caseins, and lesser whey proteins (α-lactalbumin, β-lactoglobulin) are favourable substrates for TGase, making possible the modification of several functional and structural properties of dairy products (Bönisch et al., 2007; Schorsch et al., 2000; Sharma et al., 2002). Recently, TGase was applied in ice cream manufacture as an alternative approach for producing low-fat ice cream formulations with less, or free of, stabilising agents. Elsayed Metwally (2007) investigated the effects of TGase pre-treatment of regular and low-fat ice cream mixes with varying concentrations of stabiliser. The TGase pretreatment of ice cream mixes led to a significant increase of their apparent viscosity and consistency coefficient without affecting their shear thinning behaviour. A remarkable improvement of the perceived creaminess, overall flavour and melting quality was attained though no impact on the colligative properties or perceived coarseness was observed. Rossa et al. (2011) reported that consistency coefficient of ice cream mixes was maximised using a 4U/g

TGase concentration at 56.8℃ with an incubation period of 90min. The enhancement of the apparent viscosity was attributed to the crosslinking of both κ-casein and whey proteins with the former being the most susceptible. In a successive study, Rossa et al. (2012) tested the effects of TGase treatment (4U/g, 40℃, 90min) on several techno-functional properties of ice creams of varying fat content. TGase treated ice creams exhibited an improved whipping ability, higher partial fat coalescence, better shape retention and melting resistance due to the stabilisation of the air cells via the polymerisation of caseins through covalent and intermolecular bonds (Rossa et al., 2012). The same authors have also reported that TGase treatment of ice cream mixes may lead to significant improvement of textural quality of the finished product as the protein cross-linked network may exert an important role in ice crystal growth control throughout frozen storage.

4. CONCLUSIONS

Developing and maintaining structure within frozen dairy desserts remains a challenge for the ice cream industry due to complexity of the colloidal system. Over recent years a lot of

research has been conducted to explain the impact of physical phenomena such as ice crystallisation and recrystallisation, biopolymer interactions at the water – oil and air – water interface and physical state transitions on the structure and textural stability of frozen dairy desserts. In this context, polysaccharides remain the predominant structuring and texturizing agents used in ice cream products due their ability to create structure and enhance stability via a range of complex mechanisms based on biopolymer interactions such as the formation of macromolecular networks, phase separation, water binding and cryogelation. On the other hand, consumers' demand for healthier, natural, low additive, minimally processed frozen dairy desserts has driven the ice cream industry to evaluate novel processing methods and alternative natural / functional ingredients. Health promoting ingredients such as dietary fibre, prebiotics and low glycemic index sweeteners have been used successfully for ice cream cryoprotection and texturisation due to their ability to affect mainly the colligative properties of the ice cream mixes and to provide customised texture and flavour perception by mimicking fat and sugars. Understanding biopolymer functionality at lipid – water – air interfaces has also enacted the concept of using unsaturated fatty acids in ice

cream structure development which is of particular importance for reformulation purposes.

Emerging minimal processing techniques such as ultrasound or high pressure assisted freezing have been shown as promising strategies to ensure narrow initial ice crystal distribution and consequently better control of thermodynamic instability in the case of failed storage practices. Finally, high hydrostatic pressure modification of protein induced colloidal structure and transglutaminase induced protein crosslinking can also furnish significant texturizing and shape retention benefits enabling the production of high quality ice cream with reduced use of food additives.

5. FUTURE PERSPECTIVES

Notwithstanding the extensive research that has been conducted on the colloidal aspects of frozen dairy desserts which has allowed food technologists to overcome many challenges associated with storage quality, colloidal and structure instability still remain. Current trends in the food industry for inexpensive, natural yet sustainable materials are expected to be core for the future of the ice cream industry. Utilisation of agri-food industry by-products or endemic plants for the extraction and isolation of compounds with unique technological

and functional properties seems to be a promising strategy for the development of new structuring – texturising agents for ice cream applications. Moreover, the incorporation of bioactive compounds such as probiotics, prebiotics, or phytochemicals in combination with fat reduction actives and bioactive compounds that lower glycaemic index may be necessary to meet the needs of the consumers. A significant amount of fundamental research is already being conducted in the field of functional ice creams particularly in the case of probiotic or symbiotic formulations with proven functionality under in-vivo conditions being a clear future goal. New advances in colloidal food science such as nanostructurisation (nano-emulsions, self-assembled protein microencapsulates, oleogels, double emulsions etc.) appear to be promising for the delivery of bioactive compounds to the human host without compromising storage stability and organoleptic quality. Nevertheless further work needs to be carried out in many of these areas to address stability challenges, support claim substantiation, and to enhance bioactivity. Finally, minimal processing methods such as high pressure processing, pulsed electric fields, oscillating magnetic fields, ultrasound, ohmic and microwave heating will become available for evaluation in standard production for their potential to tailor structure,

texture and sensory aspects of ice creams and finished frozen products.

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FIGURES and CAPTIONS:

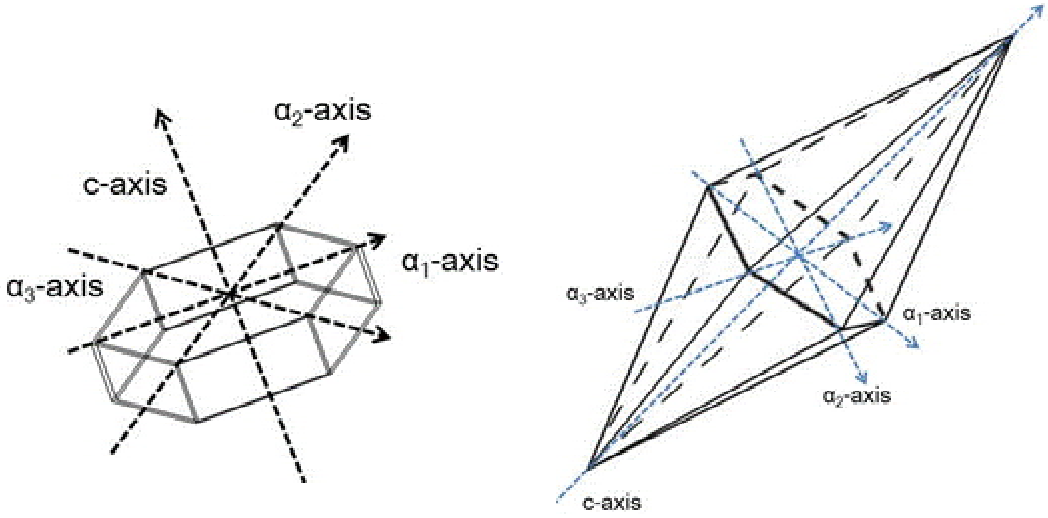

FIGURE 1: Morphological conformations of ice crystals. Lowering of aqueous system temperature induces the growth of the ice crystals along the alpha and c-axes. The presence of AFP at low concentrations favours their binding to the ice prism face leading to the formation of prism-like crystalline conformations (left). At higher concentrations of AFP in the unfrozen bulk phase, the AFP absorbed on the ice crystal prism face do not allow the diffusion of water molecules on the alpha prism surface and thus the ice crystal grows along the c-axis forming bi-pyramid crystalline structures

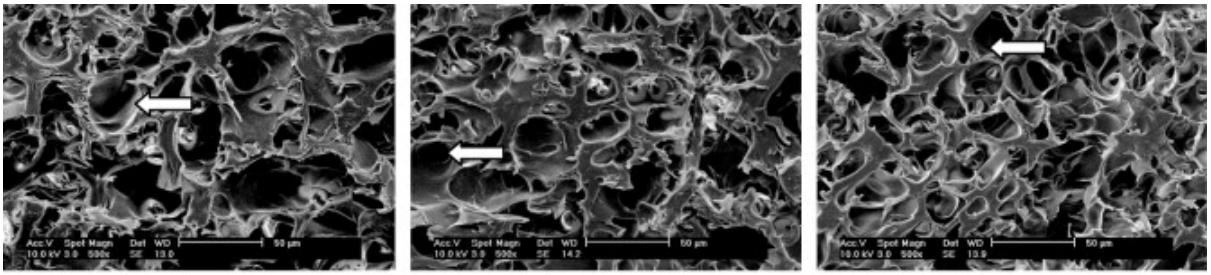

FIGURE 2: SEM micrographs of ice nucleation pattern of highly concentrated tylose (77% w/w) solution, in the absence or presence of ice nucleating bacteria - INA (*Pseudomonas syringae*). The arrows indicate the voids left after complete melting of the formed ice crystals. For all images bar scale equals to 50µm (Adopted from Zhang et al., 2009; reproduced with the permission of Elsevier)

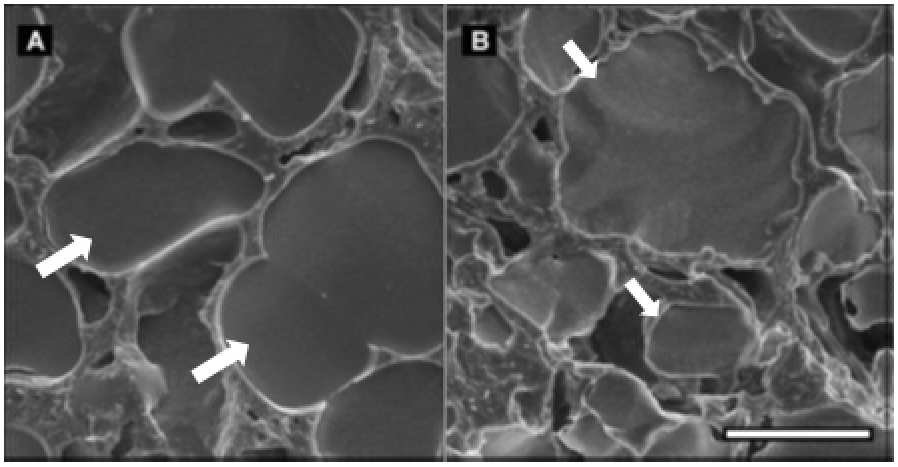

FIGURE 3: Low temperature SEM micrographs of ice cream systems in the absence (A) or presence (B) of propylene glycol monostearate. The addition of PGMS in ice cream is accompanied by the development of a curved front in the ice crystal – serum interface that leads to the increase of the surface curvature and surface free energy. The latter promotes the direct interaction of PGMS with ice crystals thereby controlling ice propagation during crystallisation and recrystallisation. Scale bar equals to 30µm. (Adopted from Aleong et al., 2008; reproduced with the permission of Wiley & Sons Inc.)

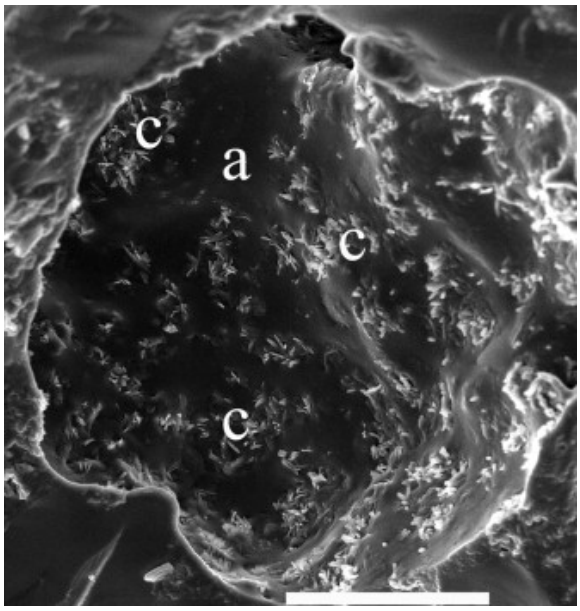

FIGURE 4: SEM migrograph of ice cream made with 40:60% of high oleic sunflower oil (HOSO) - palm kernel oil (PKO) blend. a= air cell, c = clusters of fat platelets. The presence of emulsified HOSO in the ice cream mix is associated with the formation of the fat platelet clusters as the former remains in the liquid state during the freezing whipping process. The exclusive use of PKO led to a microstructure devoid of fat platelet clusters. (Adopted from Mendez-Velasco and Goff 2012a; reproduced with the permission of Elsevier).

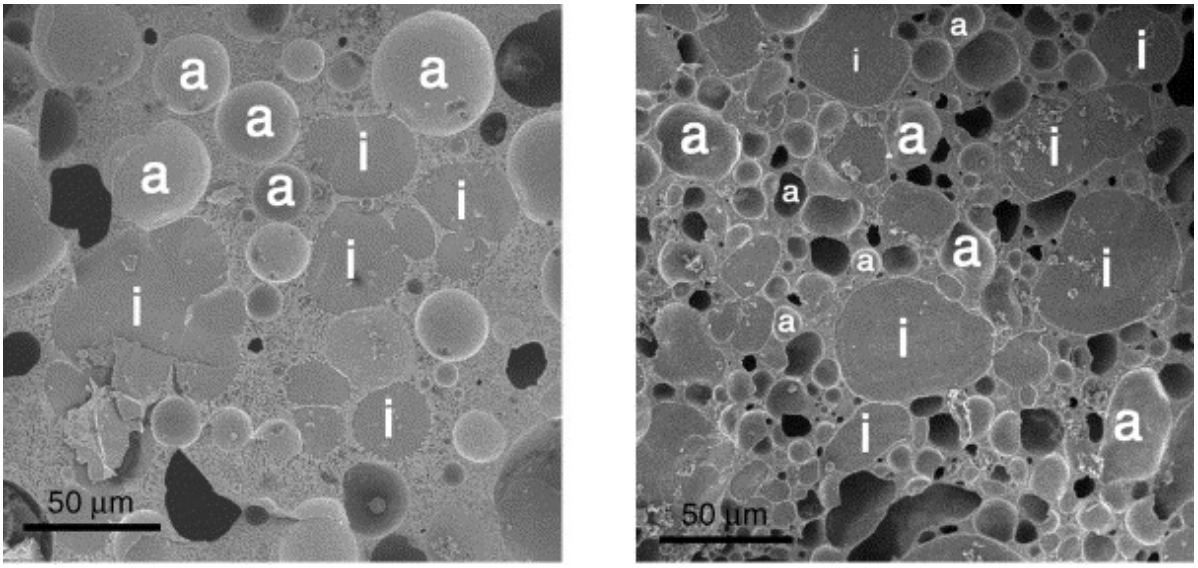

FIGURE 5: Low temperature SEM micrographs of ice cream drawn from a conventional scraped surface ice cream freezer (left) and a low temperature extruder (right). The application of high shear forces during the LTE process leads to the formation of channel like air cells structures rather than round ones. Both serum phase (area surrounding ice crystals and air cells) and air cells are finely distributed in the case of LTE treated ice cream. Ice crystals = i, air cells = a (Adopted from Eisner et al., 2005; reproduced with the permission of Elsevier)

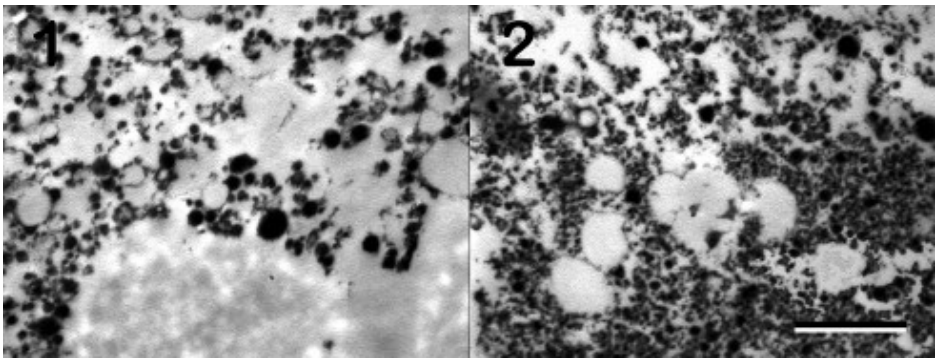

FIGURE 6: TEM micrographs of ice creams produced from untreated (left) or high hydrostatic pressure treated (right) ice cream mixes. The HHP treatment of the ice cream mixes is associated with the presence of micelles fragments or their aggregates that are densely packed. In the contrary, the control mix is mainly comprised of intact casein micelles absorbed in the fat droplets surface. (Adopted from Huppert et al., 2011; reproduced with the permission of Elsevier).